ENGLISH SUMMARY

Depression is one of the most common psychiatric diseases; indeed the prevalence of depressive symptoms has reached epidemic proportions during the last few decades. Several studies reported that depression is more prevalent in women compared to men. Although the reasons for this gender pre-dominance in depression is not understood, women show different hormonal responses that might ultimately influence behaviours and brain functions.

The core symptoms of depression include depressed mood, anhedonia (reduced ability to experience pleasure from natural rewards), irritability, difficulties in concentrating, social withdrawal (withdrawal from social contact that derives from indifference or lack of desire to have social contact) and abnormalities in appetite and sleep, the so called "neurovegetative symptoms".

Depression has shown to be comorbid with several neuropsychiatric diseases, such as schizophrenia, bipolar disorders, Alzheimer's diseases, anxiety disorders, autism spectrum disorders (ASD) and stress-related diseases. Moreover, depression often occurs during the prodromic phase of Alzheimer's disease, schizophrenia and bipolar disorders.

Diets, genetics and lifestyle contribute to the onset and progression of mental illnesses. Regarding dietary factors, Polyunsaturated Fatty Acids (PUFA) have received great attention during the last decades, particularly due to the trend towards a poor n-3 PUFA intake of modern Western diets. In this regard, in chapter 2 and 3 of the present thesis, effects of n-3 PUFA deficient and n-3 PUFA enriched diets on female rat offspring have been investigated. Our results reported that chronic exposure to n-3 PUFA deficient diet leads to highly detrimental consequences in behavioural and neurochemical parameters related to depressive- and anxiety-like symptoms. In particular, we found an increase in immobility and a decrease in swimming frequency in Forced Swimming test in n-3 PUFA deficient females, and, in the Open Field test, we showed an increase in time spent performing self-grooming and time spent in the periphery of the arena. Hence, our behavioural results showed that lifelong n-3 PUFA deficiency is able to elicit depressive- and anxiety-like symptoms in female rats. Therefore, we investigated neurochemical changes underlying these behavioural alterations. We found a significant decrease in cortical serotonin and Nerve Growth Factor in n-3 PUFA deficient females, accompanied by an increase in serotonin turnover. Moreover, in chapter 3, we showed that n-3 PUFA deficient diet led to hyperactivation of the HPA axis, in particular increase in hypothalamic noradrenaline and corticotrophin-releasing factor and

also increase in plasmatic corticosterone, accompanied by an increase in amygdaloidal noradrenaline and serotonin and an increase in glutamate and a decrease of GABA in both prefrontal cortex and amygdala. Ultimately, we found an increase in plasmatic soluble beta amyloid (Aβ) $_{1-42}$ peptide in females exposed to n-3 PUFA deficient diet.

Interestingly, soluble $A\beta_{1-42}$ peptide is receiving great importance in the development of depression, also since depression is highly comorbid with Alzheimer's disease and other neurodegenerative illnesses. Accordingly, we have previously shown that central Aβ injection is able to elicit depressive-like phenotype in male rats. Thus, in chapter 2, we reproduced for the first time the Aβ-induced depressive-like model in female rats, evaluating behavioural and neurochemical outcomes. Our results confirmed the Aβ-induced depressive-like profile also in female rats. Moreover, the Aβ-induced depressive-like profile was reversed by n-3 PUFA supplementation, indicating a possible therapeutic role of n-3 PUFA in the treatment of the burden of depressive disorders. Taken together, our data suggest that monoamine impairments, accompanied by Nerve Growth Factor alterations and HPA axis dysfunctions, might be considered important neurobiological determinants contributing to the pathogenesis of depressive-like symptoms induced by n-3 PUFA deficiency and soluble Aβ administration.

During the last decades, diagnosis in psychiatry only focused on subjective symptoms and observable signs. Although symptoms are an important starting point, genetics and neurobiology underlying these symptoms need to be deeply investigated. To achieve this purpose, animal models can be really helpful to longitudinally study behavioural alterations resembling human symptoms, and ultimately investigate the underlying neurobiology in order to unravel the etiopathogenesis. Therefore, in this thesis, we focused on depressive-like symptoms that occur in several neuropsychiatric and neurodegenerative diseases, and, using different animal paradigms and models, we tried to disentangle the neurobiological determinants behind these symptoms.

Intriguingly, in order to deeply investigate depression core symptoms in a translational way, the social sphere need to be taken into account. An important depressive-like symptom affecting the social sphere is social withdrawal. Social withdrawal, defined as lack of desire to have social contact, is an early symptom of a wide variety of neuropsychiatric diseases, including schizophrenia, Autism Spectrum Disorders (ASD) and major depression.

In chapter 4 and 5, we investigated behavioural alterations related to sociability and social withdrawal, using a behavioural paradigm called the Visible Burrow System (VBS). The VBS is a

semi-natural environment, with burrows and an open area, useful to study social dynamics that naturally occur in mixed-sex rodent colonies, firstly developed by Blanchard group. In particular we identified and validated behavioural readouts to assess sociability and social withdrawal features in C57BL/6J mice colonies, used as control strain, and two mutant lines, BTBR inbred strain and a *Pcdh9*-deficient line. The BTBR strain is a widely used strain for its similarities with human ASD deficits, such as repetitive behaviour, impaired communication and reduced social interactions, while *Pcdh9* gene Knockout (KO) mice are known to affect social behaviour in mice and may, through the core deficit in sensory processing be relevant to a wide variety of neuropsychiatric disorders, such as schizophrenia, major depression and ASD.

Our results showed that BTBR mice performed less social behaviours and have a preference for non-social behaviours compared to C57BL/6J mice in the VBS. Thus, our results reported a trend towards social withdrawal in BTBR mice, opening to a deep investigation of the underlying neurobiology that gives rise to this important symptom. Hence, our study validated the suitability of VBS as a behavioural paradigm to assess sociability and social withdrawal features. Conversely, we found no differences in terms of social behaviours and non-social behaviours among VBS colonies composed of *Pcdh9* Homozygous (HOM) and Heterozygous (HET) KO and Wild Type (WT) mice, indicating no disrupted sociability of *Pcdh9*-deficient mice when housed together with WT in the VBS. In this regard, future studies are required to better understand the HOM *Pcdh9* KO social phenotype without the presence of social stimuli. Indeed, VBS colonies formed by mixed-genotype and mixed-sex mice are considered highly social environment, and these strong social stimuli might be helpful to improve putative social deficits. In conclusion, the VBS can be used as a tool to study behavioural dysfunctions and might be further used as a behavioural paradigm to test pharmacological treatments aiming at restoring social dysfunctions that commonly occur in several neuropsychiatric disorders, such as social withdrawal.

Furthermore, in order to investigate neurobiology behind sociability and social withdrawal, in chapter 4, we analyzed GABA and glutamate content in prefrontal cortex and amygdala of C57BL/6J and BTBR colonies and we found a significant decrease of GABA and a significant increase of glutamate in both areas of BTBR mice.

Intriguingly, the decrease in GABA and the corresponding increase in glutamate in prefrontal cortex and amygdala might be responsible for the observed decrease in social behaviour and increase in social withdrawal characteristics in BTBR strain. Thus, enhancement of GABA

neurotransmission and consequent attenuation of glutamatergic tone might be a possible therapeutic strategy to treat social withdrawal symptoms that primarily occur in many neuropsychiatric and neurodegenerative diseases.

Moreover, in chapter 5, we measured GABA and glutamate levels in somatosensory cortex of *Pcdh9* colonies and we found that there were no differences in GABA content among the three genotypes in both VBS colonies and standard housing condition. Otherwise, glutamate was significantly increased only in HOM *Pcdh9*-deficient mice housed in standard cage, while no genotype differences were found in glutamate levels among VBS colonies. Hence, the glutamate increase found in HOM *Pcdh9* KO mice housed in standard cages and not found in HOM *Pcdh9* KO mice housed in VBS colonies points towards a putative beneficial effect of this highly social environment on glutamate increase induced by *Pcdh9* deletion.

In conclusion, in the present thesis, we investigated the heterogeneity underlying the neurobiology of depressive like-symptoms, that might be shared across different neuropsychiatric disorders. In this way a multifactorial perspective will be developed. Hence, in order to improve the current pharmacological approach and further develop new safe and effective treatments, the influence of social, environmental and dietary factors, together with comorbidities, need to be considered to ultimately target the correct neurobiological substrates that give rise to different depressive-like symptoms shared across various brain diseases.